

## Phenotypic Divergence and Population Variation in *Cuphea*

Abdullah A. Jaradat and Jana L. Rinke  
United States Department of Agriculture, Agricultural Research Service,  
803 Iowa Avenue, Morris, MN 56267, USA

**Abstract:** Morphological and seed-related traits in PSR23, a germplasm line selected from the inter-specific cross *Cuphea lanceolata* × *C. viscosissima* with partial seed retention, were used to quantify levels of divergence from its wild parents and levels of variation between and within its populations produced during four cropping seasons. Multivariate analyses suggest that PSR23, in response to directional selection during four cycles of sowing and harvesting, significantly diverged from its wild parents for a number of morphological and yield-related traits. Variance apportioned between PSR23 populations was dominated by plant dry weight, Leaf Area Index (LAI), branch density, capsule's major axis and seeds per capsule; however, the relatively large variance partitioned within populations for flower density, fractal dimension, 1000-seed weight and seed area (mm<sup>2</sup>) could be attributed to the low impact of directional selection for these traits in a predominantly cross-pollinated plant, growing at high density due to its indeterminate growth habit.

**Key words:** Divergence, fractal dimension, directional selection, oilseed, new crop

### INTRODUCTION

Plant populations are compelled to adapt to new ecological conditions and management practices, especially during the process of domestication (Gepts and Papa, 2002). This adaptation would result in a multitude of morphological and physiological ecotypes impacting yield and its components (Widen *et al.*, 2002). Two *Cuphea* species (*Cuphea lanceolata* and *C. viscosissima*; Lathyraceae) were emphasized as candidates for domestication and for in-depth research (Roath *et al.*, 1992) because of their oil content and composition. *C. lanceolata* is a protandrous, self-compatible, insect-pollinated allogamous diploid, with outcrossing rate >80% ( $2x = 12$ ), while *C. viscosissima* is a self-fertile, autogamous diploid ( $2x = 12$ ) and an outcrossing rate of about 30% (Knapp and Crane, 2000). In addition, the two species are inter-fertile, thus allowing for combining desired traits from both in new genotypes (White *et al.*, 1994). A partial seed retention selection (PSR23) from a cross between *C. lanceolata* and *C. viscosissima* (Knapp and Crane, 2000) is a potential new oilseed crop; its main fatty acids (i.e., capric, lauric and myristic) are used in the detergents, lubricants, cosmetics and confectionary industries (Cermak and Isbell, 2004). PSR23 was included in field trials at the North Central Soil Conservation Research Laboratory for the last six years (Sharratt and Gesch, 2004; Gesch *et al.*, 2005) with the objective of developing management practices for large

scale production. On average, seed yield, 1000-seed weight and oil content of PSR23 are about 400 kg ha<sup>-1</sup>, 2.5 g and 30.0%, respectively, with capric acid comprising most (~70.0%) of the its oil content (Gesch *et al.*, 2005 and references therein). However, PSR23 has several undesirable traits including indeterminate growth, a long maturation period, sticky leaves and seed shattering (Knapp and Crane, 2000) resulting in a very low (5.0-14.0%) harvest index when mechanically harvested (Sharratt and Gesch, 2004; Gesch *et al.*, 2005).

Architectural traits, such as plant height, branching habit and leaf area and its development during the growing season, have been shown to impact seed production in newly domesticated crops (Verdolini *et al.*, 2004). These traits may often be correlated with many measurements of plant size, especially in annuals that have indeterminate mode of reproduction and maturing seed along elongated plant modules (Cheplick, 2002) such as *Cuphea* sp. The objective of this study was to quantify the level of phenotypic divergence of PSR23 from its wild parents and estimate population variation caused by directional selection for a number of agronomic traits.

### MATERIALS AND METHODS

**Seed material:** Following terminology of the International Board for Plant Genetic Resources (IBPGR, 1991), PSR23 germplasm and wild *Cuphea* will be referred to as

populations and accessions, respectively and when combined will be referred to as entries. Seeds of PSR23, a germplasm line selected from the inter-specific cross *C. lanceolata* × *C. viscosissima* with partial seed retention, were used in greenhouse, field nurseries and replicated field experiments to carry out a series of studies during the 2002-2005 growing seasons. Random seed samples taken annually from seed harvested the previous year from field-grown PSR23 were used in a field nursery or field experiment in subsequent years. Seed of PSR23 populations produced in 2001-2004 and of ten accessions each of *C. lanceolata* (PI: 534732, 534733, 534855, 534860, 534865, 834869, 534871, 561485, 561486 and 594931) and *C. viscosissima* (PI: 534734, 534739, 534745, 534750, 534755, 534758, 534762, 534766, 534768 and 534771), used in the original cross to produce PSR23 (Knapp and Crane, 2000), obtained from the North Central Regional Plant Introduction Station, Ames, Iowa, were used in the greenhouse and field comparative studies.

**Greenhouse experiments:** Seed samples of PSR23 populations produced in the field during 2001-2003 and of 10 accessions each of *C. lanceolata* and *C. viscosissima* were planted (~ five seeds per pot) in 25 cm plastic pots during December-March of 2003 and 2004 and the same experiment was repeated in 2005 using only seed samples of PSR23 populations produced in 2001-2004. Greenhouse conditions were 20/16°C day/night temperature regime and a 16 h photoperiod. Seedlings were thinned to one per pot two weeks after germination. Five pots per entry were arranged in the greenhouse in a completely randomized design. Morphometric measurements (Table 1) were recorded on 5 single plants per entry during each year. In addition, digital images (Adamsen *et al.*, 2000) of the same single plants were taken 5 times throughout the growing

season and the final digital images were used in quantifying plant architecture and calculating a fractal dimension using Image software (Rasband, 2004). During the course of these experiments, data was recorded or measured on a total of 350 plants of PSR23 and 100 plants each of *C. lanceolata* and *C. viscosissima* in the greenhouse experiments.

**Field nurseries:** Seeds of the same entries used in the greenhouse study were hand planted directly in the field (inter and intra-row spacing of 0.75 and 0.25 m, respectively) during the 2003 and 2004 growing seasons; each entry was planted in two 5.0 m rows. Additional seeds were germinated in the laboratory then transplanted to the field to replace any missing plants during the first week after emergence. Plants were monitored for the same developmental and morphological traits as in the greenhouse experiment, in addition to seed traits and seed yield at harvest. Data was recorded or measured annually on a minimum of 10 plants of each entry.

**Field experiments:** Detailed morphological and agronomic assessments of PSR23 were made on plants, flowers, capsules and seeds in field experiments with four replicates in a randomized complete block design. Experimental plots were established in 2004 and 2005 on a Barnes-Buse loam (Barnes fine-loamy, mixed Udic Haploboroll, Buse fine-loamy, mixed, Udorthentic Haploboroll) at the Swan Lake Research Farm located near Morris, MN (45° 41' N, 95°48'W, elevation 370 m). The field site was previously in corn and soybean for the 2004 and 2005 experiments, respectively. Planting (14 kg seed ha<sup>-1</sup>; May 19, 2004 and May 17, 2005) and fertilizer application (110, 12 and 30 kg ha<sup>-1</sup> of N, P and K, respectively) were done mechanically. Each plot consisted

Table 1: Descriptive statistics, multivariate test of significance (Wilks'  $\lambda$ , a multivariate measure of phenotypic divergence between PSR23 population and its wild *Cuphea* parents) and variance components in plant, capsule and seed traits measured on five plants per year and entry each of 20 wild *Cuphea* accessions and four PSR23 populations during three (2003-2005) growing seasons

accessions and four PSR23 populations during three (2003-2005) growing seasons							
	Mean			Between entries (wild <i>Cuphea</i> and PSR23)			Within entries variance (%)
Variables	Wild <i>Cuphea</i> sp.	PSR23 populations	Wilks' $\lambda$	Variance (%)	F-value	p-value	
<b>Morphological traits</b>							
Flowers plant <sup>-1</sup>	83.00	76.00	0.134	50.4	40.30	0.00	17.4
Main branches plant <sup>-1</sup>	7.50	6.30	0.097	75.1	19.00	0.00	15.1
Plant height (cm)	105.00	98.00	0.109	63.7	2.56	0.01	12.9
Plant dry weight (g)	9.30	8.90	0.107	62.3	7.03	0.00	26.3
Leaf area index (m <sup>2</sup> m <sup>-2</sup> )	6.90	6.50	0.295	39.2	4.94	0.05	25.4
Average				58.1			19.4
<b>Capsule and seed traits</b>							
Mature capsules per plant	59.00	56.00	0.128	59.5	4.95	0.06	24.8
Seeds per capsule	12.20	10.40	0.165	56.7	6.60	0.01	24.3
1000-seed weight (g)	2.25	2.91	0.089	68.8	6.89	0.01	19.6
Seed area (mm <sup>2</sup> )	6.03	7.92	0.024	53.7	22.80	0.01	26.4
Seed circularity (%)	0.85	0.88	0.563	25.2	2.04	0.80	39.5
Average				52.8			26.9
Seed yield (g plant <sup>-1</sup> )	0.61	0.73	0.209	36.8	12.80	0.05	28.3

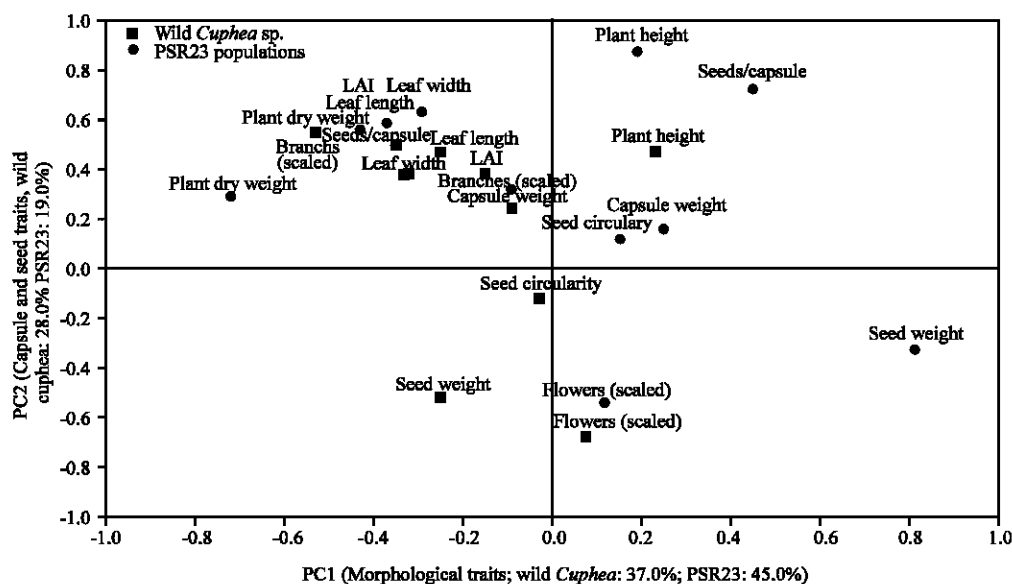


Fig. 1: Loadings on and percent variation explained by the first two principal components in the multivariate analyses of plant, capsule and seed traits of 20 wild *Cuphea* accessions and populations of the *Cuphea* germplasm line PSR23 averaged over three (2003-2005) growing season

of six rows (6 m long and 60 cm row spacing). Weed control was carried out to ensure a weed-free stand. For the purpose of this study, six single plants were randomly sampled at physiological maturity from each replicate for detailed morphological and agronomic evaluation and to assess the range of variations in PSR23 (Table 1 and Fig. 1). Number of flowers and capsules were monitored five times during 2004 and 2005 on plant<sup>-1</sup> and m<sup>-2</sup> basis, respectively and seed dry mass data was measured at harvest using the inner four rows in each plot.

**Digital imagery:** Digital imagery (Adamsen *et al.*, 2000) and analysis procedures (Foroutan-pour *et al.*, 2000; Rasband, 2004) were used to capture and measure morphological traits of individual plants, capsules and seeds produced in the greenhouse, nursery and field experiments (Table 1). Images of the plants in the field were taken five times during the growing season to estimate green area by positioning the FirstGrowth camera above the top of the plant canopy so that the same spot is viewed in each plot over time. Three replicates, each of 100 random seeds from PSR23 produced during four cropping seasons and a similar number of seed from wild *Cuphea* accessions were manually positioned on the platform of a CanoScanLide 30 scanner ensuring seeds were totally separated from each other (and verified by the number of objects generated by the scanning software), then scanned. During the 2004 and 2005 cropping

seasons, approximately 200 mature random capsules of PSR23 were weighed individually, then seed weight and seed number per capsule were obtained. A scale (in mm for seeds and capsules, or in cm for whole plants) was attached to each object (plant, capsule or seed lot) and the generated image was saved as a digitized 8-bit gray image with a resolution of 300 pixels. For each scanned image, all measurements were adjusted based on the pixel-to-mm or pixel-to-cm conversion scale.

**Statistical analyses:** One way multivariate analyses of variance (MANOVA) for evaluating population or accession differences were performed using Wilks'  $\lambda$  as the test statistic. Patterns of morphological similarities or dissimilarities were analyzed by multivariate statistical methods (Hair *et al.*, 1998). A dimension reduction and perceptual mapping statistical procedure (Hair *et al.*, 1998; Rohlf, 2000) was employed to reduce the dimensionality of a matrix of wild *Cuphea* accessions and PSR23 populations based on all morphological and yield-related traits measured on seeds, capsules and plants. The Principal Components (PC) option in the Nonlinear Iterative Partial Least Squares (NIPALS) module and Canonical Discriminant (CD) analyses were used to analyze morphometric patterns of individual plants within each population, accession and year and to quantify possible differences between wild *Cuphea* accessions and PSR23 populations and between PSR23 populations evaluated during the 2002-2005 cropping seasons.

Canonical discriminant analysis was used to quantify multivariate relationships between wild *Cuphea* accessions and PSR23 populations and between PSR23 populations based on their morphology and yield-related traits. The eigenvectors resulting from PC analyses and the standardized discriminant function coefficients resulting from CD analyses were used to identify the traits that most significantly contribute to classifying populations and accessions. The fractal analysis procedure employed the box count concept as outlined by Foroutan-Pour *et al.* (2000), where the fractal dimension,  $D_0$ , is constrained to be in the range of  $1.0 \leq D_0 \leq 2.0$ . A value of 1.0 indicates that the image is completely differentiable and that of 2.0 indicates that the image is very rough and irregular.

Phenotypic divergence was assessed by Squared Mahalanobis Distances ( $D^2$ ) between and within wild *Cuphea* accessions and PSR23 populations. Covariance matrices based on the data set in Table 1 of wild *Cuphea* accessions and PSR23 populations were compared with the Common Principal Components (CPC) technique (Flury, 1988; Rohlf, 2000). CPC tests whether two or more covariance matrices have completely unrelated structures, or whether the matrices share one or more principal components (eigenvectors). The jump-up approach (Phillips *et al.*, 2001; Rohlf, 2000) was used to determine the highest point in the Flury hierarchy at which accumulated differences in the matrices became significant and used the model immediately below as the best fitting model for the observed covariance matrices. In addition, similarity matrices, based on plant, capsule and seed traits, of wild *Cuphea* accessions and PSR23 populations were used, with 1000 permutations, to test whether each pair of these entries are independent (Mantel, 1967). Statistical analyses were performed using the relevant modules in the software packages STATISTICA 7.1 (StatSoft Inc., 2005a) and NTSYS-pc (Rohlf, 2000).

## RESULTS

**Patterns of phenotypic variation:** Mean values for 12 traits measured on plants, capsules and seeds sampled from greenhouse and field nursery experiments are shown in Table 1. Phenotypic divergence of PSR23 from its wild parents is supported by the significance of the multivariate test statistic (Wilks'  $\lambda$ ) and percent and level of significance (p-value) of variance apportioned between entries. On average, 58.1 and 52.8% of total variance in morphological and capsule and seed traits was apportioned between entries, respectively. The respective values for within entries were 19.4 and 26.9%. Finally,

difference in seed yield per plant between entries was significant, with 36.8 of its variance apportioned between entries. Variation in morphological traits was dominated, in a decreasing order, by branch density (main branches per plant), plant height and plant dry weight; whereas variation in capsule and seed traits was dominated, in decreasing order, by 1000-seed weight, mature capsules per plant and seeds per capsule. However, variation in mature capsules per plant was marginally significant; whereas no significant differences were detected in seed circularity. Seed yield per plant differed significantly among entries, however, less than 40% of the variation was apportioned between entries.

### Multivariate analyses of phenotypic variation:

Eigenvectors in the first two principal components (PCS; Fig. 1) and standardized discriminant coefficients in the first two Canonical Discriminant (CD) functions in the multivariate analyses of plant, capsule and seed attributes (Fig. 2) identified major differences and a few similarities between wild *Cuphea* accessions and PSR23 populations grown in the greenhouse and field nurseries. The first two PCS (Fig. 1) accounted for almost the same amount of variation (~ 65.0%) in wild *Cuphea* accessions and PSR23 populations; however, with slightly different percent variances explained by and major differences in trait loadings. Of particular significance are the different loadings of plant height, plant dry weight, leaf dimensions, 1000-seed weight and seeds per capsule between wild *Cuphea* accessions and PSR23 populations.

The Fig. 1 shows that LAI is positively correlated with branch density, plant dry weight and negatively with plant height and flower density. On the other hand, plant dry weight is associated more with branch density than with plant height, although all three variables loaded positively on PC2. On the other hand, seed circularity is decoupled from both seed weight and seeds per capsule. All three variables have relatively small loadings on both PCS and seed weight is negatively associated with flower density in wild *Cuphea* accessions; however, these two variables have positive loadings on PC1 in PSR23 populations.

The first and second canonical discriminant functions were highly significant ( $p < 0.01$ ) and accounted for 70 and 15 of total variance in wild *Cuphea*, respectively and 53 and 29% of total variance in PSR23 populations, respectively (Fig. 2). In wild *Cuphea* accessions, the first canonical discriminant function was dominated by LAI, seeds per capsule and seed circularity, whereas flower density and plant dry weight dominated the second discriminant function. The respective dominant variables for PSR23 populations on the first canonical discriminant

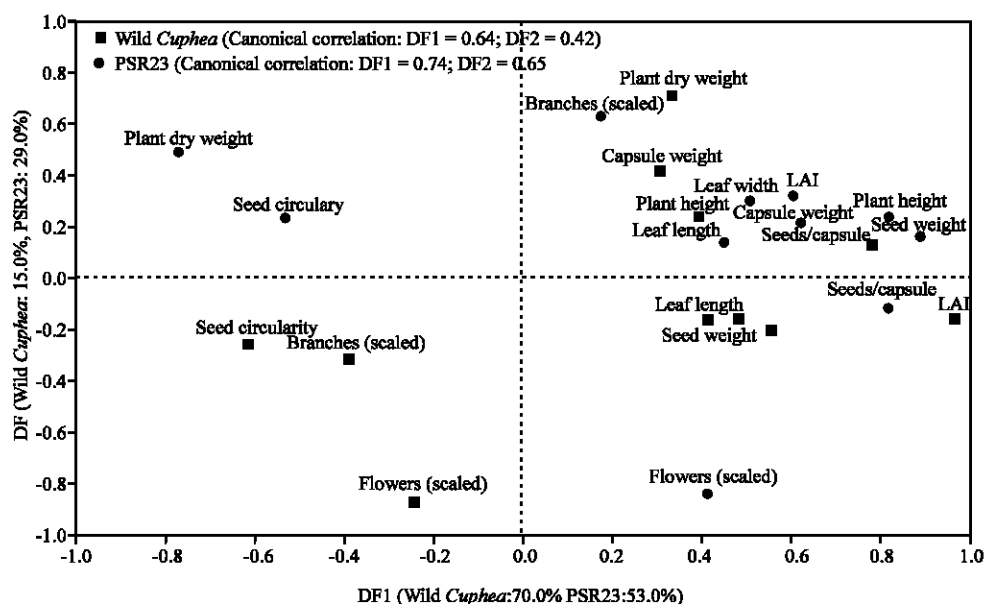


Fig. 2: Standardized discriminant coefficients of the first two discriminant functions in the multivariate analyses of plant, capsule and seed attributes of 20 wild *Cuphea* accessions and populations of the *Cuphea* germplasm line PSR23 averaged over three (2003-2005) growing season

Table 2: Number, minimum and maximum and percent significant Squared Mahalanobis Distances ( $D^2$ , indicating phenotypic divergence between entries) between wild *Cuphea* accessions and PSR23 populations based on 10 morphological traits measured on five plants per accession or population in each of three (2003-2005) growing seasons

Pairwise comparison	No. pair-wise comparisons	No. of significant ( $p < 0.05$ ) $D^2$	Minimum-maximum $D^2$	Percent significant $D^2$
Between <i>C. lanceolata</i> accessions	45	26	38.5-72.9	58.0
Between <i>C. viscosissima</i> accessions	45	17	24.2-47.5	38.0
Between <i>C. lanceolata</i> and <i>C. viscosissima</i> accessions	190	165	88.9-137.8	86.8
Between PSR23 populations	6	2	23.6-48.8	33.3
Between PSR23 populations and <i>C. lanceolata</i> accessions	91	35	45.4-82.9	38.5
Between PSR23 populations and <i>C. viscosissima</i> accessions	91	83	83.3-125.9	91.2

function were 1000-seed weight, seeds per capsule, plant height and plant dry weight and on the second discriminant function were flower density, branch density and plant dry weight. Canonical correlation values (range from 0.42 to 0.74, Fig. 2) indicate that the canonical varieties can explain the differences between wild *Cuphea* accessions and PSR23 populations.

The Squared Mahalanobis Distances ( $D^2$ ) between centroids of wild *Cuphea* accessions and PSR23 populations (Table 2), derived from the canonical discriminant analyses, provided a quantitative assessment of the phenotypic divergence between and within these entries. Phenotypic divergence between *C. lanceolata* and *C. viscosissima* was the largest, followed by divergence between PSR23 populations and *C. viscosissima* accessions, whereas the phenotypic divergence between PSR23 populations was the smallest. The range and percent significant  $D^2$  values suggest that more similarities exist between PSR23 and *C. lanceolata* as compared to those between PSR23 and *C. viscosissima*

for these traits. The largest percent significant  $D^2$  values were expressed by PSR23 and *C. viscosissima* (91.2%), followed by *C. lanceolata* and *C. viscosissima* (86.8%); whereas the lowest (33.3%) was expressed by PSR23 populations.

The Common Principle Components (CPC) analyses (Table 3) involving all co-variance matrices, derived from plants grown in greenhouse and field nurseries, provided further insights into the relationships between and within wild *Cuphea* accessions and PSR23 populations. The hierarchical comparisons between their co-variance matrices averaged over two years suggests that they share up to five principal components and that CPC5 is the best fitting model that describes the multivariate relationship between entries. Furthermore, the non-significant matrix correlation coefficient ( $r = 0.56$ ,  $p = 0.07$ ) based on 1000 permutations supports this relationship and indicates that there are more differences than similarities between these entries. On the other hand, the differences between PSR23 populations and

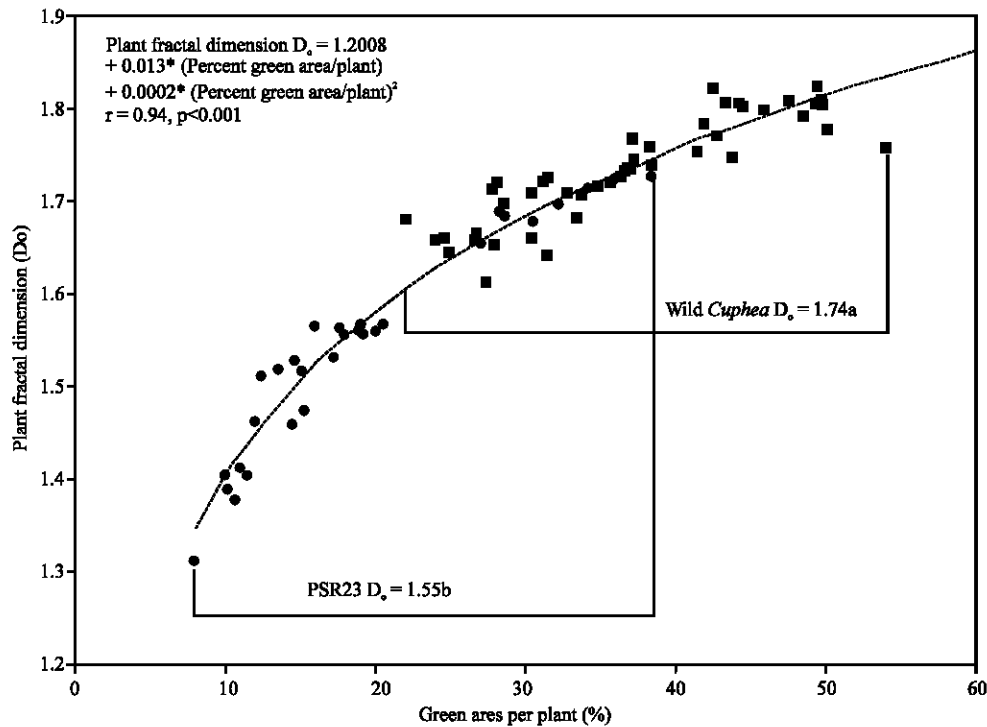


Fig. 3: Plant fractal dimension of wild *Cuphea* sp. and the germplasm line PSR23 as a function of percent green area per plant digitally measured five times during the growing season on plants averaged over three (2003-2005) growing seasons

Table 3: Hierarchical comparisons among co-variance matrices based on 12 morphological traits (shown in Table 1) measured on five plants each of 20 wild *Cuphea* sp. (*C. lanceolata* and *C. viscosissima*) accessions and of four PSR23 populations and among four PSR23 populations produced in 2002-2005

Comparison	Year seed was produced		Best fitting model (p-value)	Matrix correlation coefficient	p-value
<i>C. lanceolata</i> -PSR23			CPC5 (0.28)	0.56	0.07
<i>C. viscosissima</i> -PSR23			CPC2 (0.22)	0.35	0.14
PSR23-PSR23	2002	2003	CPC6 (0.32)	0.78	0.01
	2002	2004	CPC8 (0.44)	0.82	0.01
	2002	2005	CPC7 (0.30)	0.71	0.05
	2003	2004	CPC8 (0.50)	0.89	0.01
	2003	2005	CPC9 (0.48)	0.82	0.01
	2004	2005	CPC7 (0.39)	0.75	0.03

*C. viscosissima* accessions are much larger based on the very few CPC (only two) and the non-significant ( $r = 0.35$ ,  $p = 0.14$ ) matrix correlation coefficient. Hierarchical comparisons between co-variance matrices of PSR23 populations evaluated in greenhouse (2003-2005), field nurseries (2003-2004) and field experiments (2004-2005) showed no clear temporal trend towards more or less multivariate similarities between these populations. The best fitting model for pairwise comparisons ranged from CPC6 to CPC9, with all matrix correlation coefficients being highly significant.

**Fractal dimension ( $D_o$ ) analysis:** Skeletal images of plants grown in greenhouse and field nurseries and experiments, when analyzed using Image software, revealed a

large level of complexity as indicated by the fractal dimension ( $D_o$ ) values (Fig. 3). The fractal dimension of a single plant can be predicted with high accuracy ( $r = 0.94$ ;  $p < 0.001$ ;  $R^2 = 88.4$ ) as a function of percent green area, regardless of its branching pattern. PSR23 populations differed significantly ( $p < 0.05$ ) in their fractal dimension estimates ( $D_o = 1.55$ ) from wild *Cuphea* accessions ( $D_o = 1.74$ ), although there was some overlapping in these estimates. The lower ( $\sim 1.3$ ) and upper ( $\sim 1.7$ )  $D_o$  limits for PSR23 populations suggest that these plants are highly branched and they develop a complex vegetative growth pattern whether grown in the greenhouse or in the field. The respective values for wild *Cuphea* accessions ( $\sim 1.6$  and  $\sim 1.8$ ) also strongly suggest that the branching and foliage development are even more complex as compared to PSR23 populations.

## DISCUSSION

All of our existing major crop species have been through a continual process of domestication and improvement since the beginnings of agriculture more than 10,000 years ago (Gepts and Papa, 2002). However, research aimed at the domestication of new species, especially potential novel oilseed crops, is being hampered by lack of funding (Murphy, 1999) and by the many agronomic problems faced by new oilseed crops (Graham, 1989; White *et al.*, 1994; Verdolini *et al.*, 2004; Baye and Becker, 2005).

Inter-specific hybridization, as an important mechanism for generating evolutionary novelty in plants (White *et al.*, 1994), followed by mass selection (Knapp, 1990; Knapp and Crane, 2000) succeeded in producing PSR23 as a potentially valuable germplasm for the development of industrial oilseed crop. The original germplasm used in developing PSR23 (i.e., *C. lanceolata* and *C. viscosissima*), although inter-fertile, differed widely in a number of morphological traits including plant height and flower and seed traits (Hirsinger and Knowles, 1984).

Multivariate differences between wild *Cuphea* accessions and PSR23 populations (Table 1) suggest that agricultural practices, including sowing and harvesting cycles, has significantly influenced morphological divergence of PSR23 from its wild parents at the plant and seed levels. Directional selection is known (Verdolini *et al.*, 2004; Annicchiarico, 2006) to affect morphological traits of plant populations in environments that are very different for climatic conditions and agronomic practices from those under which the plant germplasm was selected. The large variation for some agronomic traits in PSR23 may be attributed to the low impact of directional selection for such traits in the predominantly cross-pollinated PSR23 plants, grown at high density (Annicchiarico, 2006).

Traits that might have been impacted by directional selection and expressed in PSR23, are those associated with growing in greater density than in the wild such as plant height and leaf size (Gepts and Papa, 2002), thus creating fierce competition among plants within the population for resource acquisition (Cheplick, 2002). The most important traits that helped explain patterns of variation in PSR23 were plant height, plant dry weight, 1000-seed weight and seed number per plant (Fig. 2, 3). Recent experimental evidence (Baye and Becker, 2005) suggests that human effects on plant evolution after domestication may result from directional selection through agricultural practices which are expected to encourage the emergence of new genotypes and then by stabilizing selection to maintain the newly domesticated population. Furthermore, unconscious selection in the

presence of relatively large intra-populations variation (Gepts and Papa, 2002) and derived from sowing-harvesting cycles may have impacted certain traits in PSR23 such as seed retention and larger seed size as compared to the wild species. Similar findings were reported for Verdolini *et al.* (2004) and *Vernonia galamensis* (Baye and Becker, 2005).

Matrix comparisons between wild *Cuphea* accessions and PSR23 populations (Table 3) suggest the existence of extensive divergence in the covariance matrices and also indicates changes in the principal components structure in addition to large changes in eigenvalues (Fig. 1). CPC models (Table 3) were specific to certain pairwise comparisons, ranging from CPC2 to CPC9. These results can be attributed to two major patterns in the covariance matrices (Widen *et al.*, 2002). The first: different populations or accessions showed high or low co-variances for different traits, especially plant height, plant dry weight and 1000-seed weight. The second: other traits, especially LAI, disproportionately have large influence on differences in the mean covariance and as depicted by their loadings on the first two principal components (Fig. 1) and discriminant functions (Fig. 2). These results support the suggestion that directional or natural selection has caused large, idiosyncratic changes in the principal component structure and that some of the changes can be attributed to shifts in the mean phenotype (Widen *et al.*, 2002).

Variation in fractal dimension between entries (Fig. 3) provided some insight into their branching patterns. Lower and upper limits of  $D_0$  in PSR23 (1.3-1.7) reflect a highly branched architecture and attests to the complex vegetative growth pattern plants develop whether grown in the greenhouse or in the field. Nevertheless, PSR23 populations and wild *Cuphea* accessions ( $D_0$  range from 1.6 to 1.8) differed significantly in their  $D_0$  estimates; and the branching and foliage development of wild *Cuphea* accessions were even more complex. In general, as the indeterminate *Cuphea* plant grew and became more branched,  $D_0$  increased and the architecture became more complex in order to acquire light resource (Foroutanpour *et al.*, 2000; Cheplick, 2002). Fractal analysis may provide new avenues for understanding the functional implications of branching patterns in relation to optimum resource acquisition and allocation, flowering, capsule set and yield (Egli, 2005).

## CONCLUSIONS

We quantified the level of variation in the *Cuphea* germplasm line PSR23 as a potential oilseed crop and its divergence from its wild parents based on seed, capsule and plant traits. Several cycles of sowing and harvesting

significantly influenced morphological divergence of PSR23 from its wild parents and resulted in generating large variation at the plant, capsule and seed levels. The most important traits that helped explain patterns of variation in PSR23 were plant height, plant dry weight, 1000-seed weight and seed number per plant. Fractal analysis of whole plants provided new avenues for understanding the functional implications of branching patterns in relation to optimum resource acquisition and allocation, flowering, capsule set and yield. This information is of value for *Cuphea* breeders and agronomists in their quest to fully domesticate this potential oilseed crop.

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